Plant Synthesis: Garlic

Used to synthesize gold nanoparticles ranging from 1-10nm.

Protocol:

Garlic aqueous plant extraction

1. Peel garlic cloves and wash thoroughly with tap water.
2. Rinse garlic cloves with ionized water, then dry them with a paper towel.
3. Weigh out approximately 3 g of garlic cloves. Cut the cloves with scissors or a knife into smaller pieces in order to more accurately weight to 3 g.
4. Grind the weighed 3 g of garlic cloves using a mortar and pestle.
5. Filter the ground garlic using a Whatman filter 1 paper and glass funnel into a 100 mL Erlenmeyer flask with 30 mL of MilliQ water. Set up a vacuum apparatus to aid transfer of water-garlic extract into the flask.
6. The garlic extract solution collected in the flask is then passed through a 0.45 micron Whatman filter 1 paper-covered funnel into a new clean flask to further filter the liquid extract. This is repeated twice.
7. Store the garlic extract solution in glass (Duran) bottles at 4°C in fridge.

- Do NOT place the garlic extract solution in a freezer (at -20°C or -80°C) - freezing the solution will alter the active ingredient in the garlic extract and decrease yield of nanoparticles during synthesis

Synthesis of gold nanoparticles using aqueous garlic extract solution

1. Preset water double boiler to 95°C. Set up the double boiler in a fume hood using a hot plate and a large beaker filled with tap water. Use a mercury thermometer to monitor the temperature of the water bath.
2. Make 20 mL of a 1% garlic extract solution.

- Using a graduated cylinder, measure 19.8 mL of ddH2O and pour into a 100 mL beaker
- Micropipette 200 uL (0.2 mL) of garlic extract solution into water in the beaker
- Place the beaker on a stir plate and add a magnetic stir bar to the beaker to allow the mixture to stir and become homogeneous

3. Prepare 67 uL of 75 mM HAuCl4 solution.

A. Weigh out 2.55 mg (0.00255g) of chloroauric acid trihydrate (HAuCl4) solid on a weigh-boat.

- CAUTION: oxidizes quickly - do not expose to air for longer than required
- Do NOT use metal scoopula - use plastic or wood to transfer the solid
- LIGHT SENSITIVE - use for short duration under light conditions
- Note: chloroauric acid is pricey - try not to be wasteful!

B. Using a micropipet, quickly add ~100 uL of the ddH2O from the flask to the crystals of chloroauric acid trihydrate on the weigh-boat. This should dissolve the solid to generate a yellow mixture on the weigh-boat.

4. Use the micropipette to transfer the 67 uL of chloroauric acid solution to the 20 mL garlic solution in the beaker. Stirring should be continuous during this process to ensure dispersion of the HAuCl4.

5. Use pH paper to determine the pH of the garlic-HAuCl4 solution.

6. Add NaOH (40%) very slowly dropwise to the mixture in the beaker until a pH ~10 with stirring. Wait several seconds between the addition of each drop of NaOH. This gives time for the NaOH to equilibrate in the mixture and stabilize the overall pH of the solution.

- A basic pH is required to allow for the nanoparticle synthesis reaction to occur.

7. Fix the beaker containing the mixture in the makeshift water bath by immersing the lower half of the beaker in the heated water.
    - Use the clamp of a ring stand in the fume hood to stabilize the setup.
    - Nanoparticle synthesis occurs at this step - a color change of solution to pink or purple indicates nanoparticle formation
8. Allow up to 20 minutes for nanoparticle synthesis reaction
    - At approximately 7-12 mins, if a significant color change is seen, remove the beaker from the water bath
    - Keeping the beaker for longer in the water bath past color change may cause nanoparticle aggregation and generation of oversized gold particles
9. In a small sealed vial wrapped in aluminum foil, the nanoparticle solution can be stored in a 4°C fridge.
    - Covering the vial in aluminum foil prevents the nanoparticles from being exposed to light which will prolong the shelf-life and integrity of the particles

Source: Rastogi, L., & Arunachalam, J. (2013). Green synthesis route for the size controlled synthesis of biocompatible gold nanoparticles using aqueous extract of garlic (allium sativum). *Advanced Materials Letters*.

Plant Synthesis: Cabbage

Used to synthesize silver nanoparticles ranging from 15-30nm.

Protocol:

Cabbage aqueous plant extraction

1. Cut cabbage into small fine, thin strips and weigh out 25 g on a scale.
   - Do not mince the cabbage, as this could cause loss of the juices of the vegetable which contains the active ingredients for later nanoparticle synthesis.
   - Cutting the cabbage smaller without squeezing the vegetable too much allows for increased surface area of the vegetable to be exposed for extraction of the vegetable's juices - this is optimal for production of cabbage extract
2. Wash the cut cabbage thoroughly with dH2O.
3. Dry the washed cabbage with paper towels.

Perform all of the following steps in a FUME HOOD:

4. Using a 200 mL graduated cylinder, measure out 150 mL of ddH2O.
5. Pour the 150 mL of water into a clean 250 mL beaker and place the beaker on a hot plate. Allow the water to reach a boil on the hot plate.
   - To quicken the boiling of the water, a watch glass can be placed atop the opening of the beaker.
6. Place the pieces of washed and cut cabbage into the boiled water in the beaker.
7. Boil the cabbage and water mixture for 5-10 mins.
8. While the cabbage is boiling, take a new clean 250 mL Duran bottle and cover its surface in aluminum foil so its contents can be protected from light exposure.
9. Place a funnel with a Whatman paper 1 filter coating its opening, at the mouth of the aluminum foil-covered Duran bottle.
10. While wearing the proper oven mitts, pour the boiled cabbage solution into the Duran bottle through the filter-coated funnel to remove the chunks of remaining cabbage from the cabbage extract solution. Perform additional filtrations with 0.45 um and/or 0.22 um filters if desired. (additional filtrations have shown to narrow the range of potential sizes of silver nanoparticles to within 30-50 nm). Cap the bottle once the transfer is complete.
11. The cabbage extract can be stored at 4°C in the Duran bottle.
    - Do NOT place the cabbage extract solution in a freezer (at -20°C or -80°C) - freezing the solution will alter the active ingredient in the cabbage extract and decrease yield of nanoparticles during synthesis
    - Keep the cabbage extract solution **away from light** to prevent breakdown of reactive species in solution.

Silver (spherical) nanoparticle synthesis using cabbage aqueous plant extract

1. Gather the cabbage extract solution from storage in the 4°C fridge.
2. Prepare 90 mL of 1 mM silver nitrate (AgNO3) solution in a 150 mL beaker/Erlenmeyer flask.
   - On a scale, weigh out 0.01529 g (15.29 mg) of AgNO3 solid
   - Using a 100 mL graduated cylinder, measure out 90 mL of ddH2O
   - In the ***fume hood***, pour the water into a 150 mL Erlenmeyer flask coated in aluminum foil - wrapping the flask in foil will prevent the solution from being exposed to light as the silver nitrate is light sensitive
   - Add the measured quantity of AgNO3 solid to the flask with water in the fume hood
   - Seal the opening of the flask with Parafilm and mix the solution by inversion

3. Using the same graduated cylinder from Step 2, measure 10 mL of cabbage extract solution.
4. Pour the measured cabbage extract into the Erlenmeyer flask in the fume hood containing the 1 mM AgNO3 solution.
5. Cover the opening of the flask with Parafilm then invert the flask to mix the solution.
6. Let the solution in the flask sit at room temperature for 12 hrs to allow nanoparticle synthesis.
    - After 3 hours, the solution should undergo a color change to **dark brown** or **honey brown** indicating nanoparticle formation
    - To view the color of the solution, peel back some of the aluminum foil to view the contents of the flask - once done viewing, cover the flask properly to limit the solution's exposure to light
7. Following nanoparticle synthesis, the solution can be stored at room temperature.

Source: Tamileswari, R., Nisha, M. H., & Jesurani, S. S. (2015). Green Synthesis of Silver Nanoparticles using Brassica Oleracea (Cauliflower) and Brassica Oleracea Capitata (Cabbage) and the Analysis of Antimicrobial Activity, *4*(04), 1071-1074.

Plant Synthesis: Aloe Vera

Used to synthesize a variety of shapes including nanotriangles.

Protocol:

1. In a clean 100 mL beaker wrapped with aluminum foil to prevent light exposure, add a chosen volume of aloe vera plant extract:
    - Varying volumes ranging between 0.5mL - 4mL of aloe vera plant extract can be used to synthesize gold nanoparticles
    - The greater the volume of aloe vera extract used (eg. 1mL) the smaller the gold nanotriangles that will be generated
    - To synthesize nanoparticles to target size (less than 100nm), use 4mL of aloe vera extract
2. Measure 6-9.5 mL of ddH2O (depending on the desired amount of garlic extract) using a graduated cylinder. Add this to a 25-40mL beaker.
3. Weigh out 0.00204g (2.0387mg) of chloroauric acid solid
    - CAUTION: oxidizes quickly - do not expose to air for longer than required
    - Do NOT use metal scoopula - use plastic/Teflon scoopula to transfer the solid
    - LIGHT SENSITIVE - use for short duration under light conditions
    - RAPIDLY add the measured chloroauric acid into the ddH2O in the 25-40mL beaker
    - Parafilm the top of the beaker to seal it
    - Add a stir bar and place the beaker on a stir plate to dissolve the chloroauric acid
4. Using a 10mL graduated cylinder, measure between 0.5-4mL of garlic extract and add it to the chloroauric acid solution in the beaker to bring the volume up to 10mL - continue stirring throughout
5. Remove the beaker with solution from the stir plate and leave it covered with Parafilm in the dark or covered in aluminum foil (minimize light exposure of solution) in the biosafety cabinet for 30hr (reduction of gold occurs during this period)
    - At ~5hr in, remove the aluminum foil to check for a color change. The solution should appear **dark brown**/**purple in color** - indicator of nanoparticle synthesis (may test for color formation using UV spectrophotometer)
6. The nanoparticle solution can be stored between 4C - 20C
    - At temperature lying outside the range of 4-20C, the nanoparticles aggregate and become unstable
    - Storage of the solution in the 4C fridge is optimal
    - Store AWAY from direct sunlight
    - Do NOT freeze

Source: Chandran, S. P., Chaudhary, M., Pasricha, R., Ahmad, A., & Sastry, M. (2006). Synthesis of gold nanotriangles and silver nanoparticles using Aloe vera plant extract. *Biotechnol. Prog.*, *22*(2), 577-583.